Fig. 1A

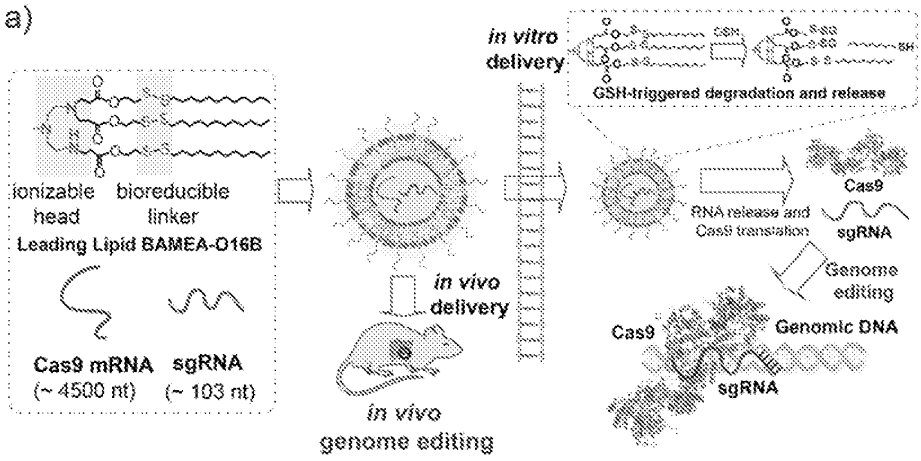

a)
in vitro delivery
GSH-triggered degradation and release
ionizable head
bioreducible linker
Leading Lipid BAMEA-O16B
Cas9 mRNA (~ 4500 nt)
sgRNA (~ 103 nt)
in vivo delivery
in vivo genome editing
RNA release and Cas9 translation
Cas9
sgRNA
Genome editing
Genomic DNA

Fig. 1B

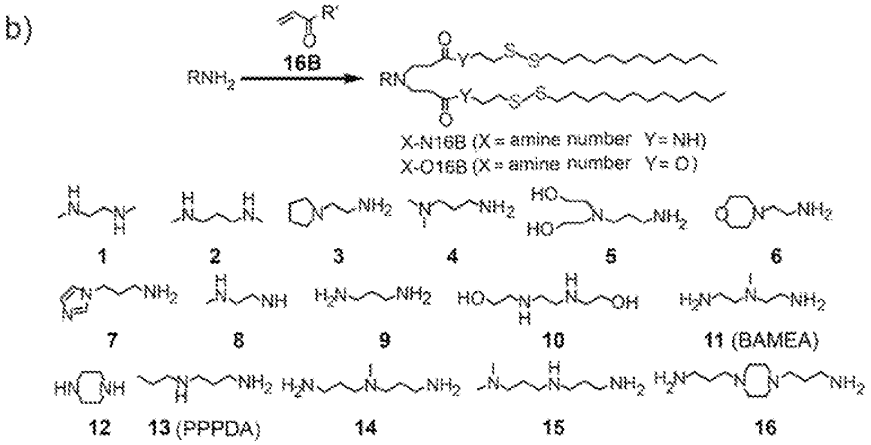

b)
16B
X-N16B (X = amine number Y = NH)
X-O16B (X = amine number Y = O)
1
2
3
4
5
6
7
8
9
10
11 (BAMEA)
12
13 (PPPDA)
14
15
16

Fig. 2A

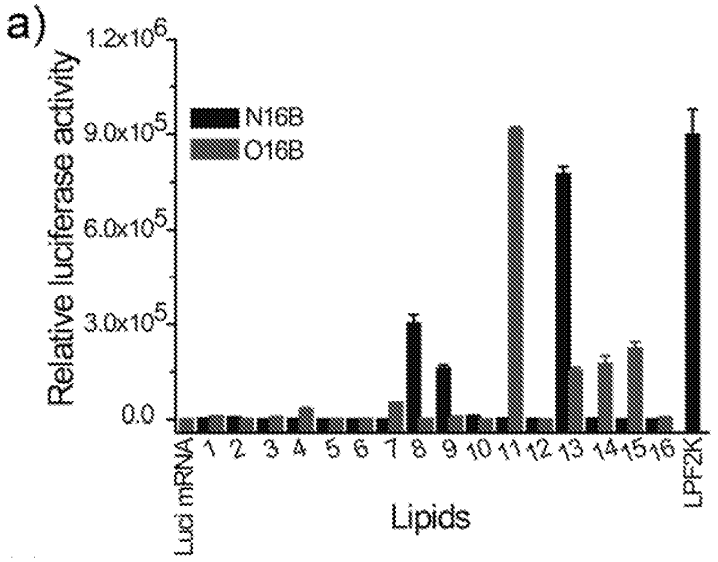

a)
Relative luciferase activity
1.2x10^6
9.0x10^5
6.0x10^5
3.0x10^5
0.0
N16B
O16B
Luci mRNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
LPF2K
Lipids

Fig. 2B

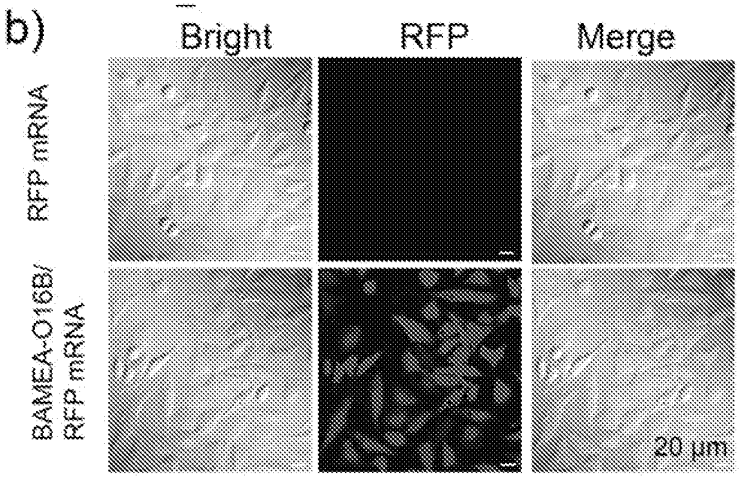

b)
Bright
RFP
Merge
RFP mRNA
BAMEA-O16B/
RFP mRNA
20 μm

Fig. 2C

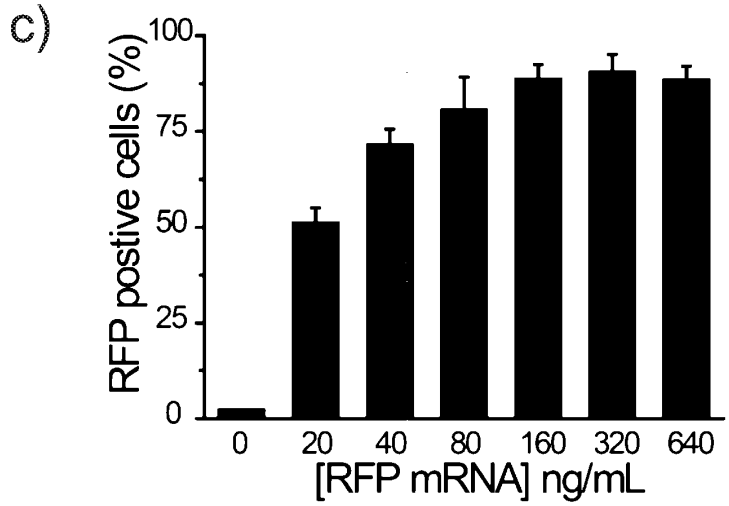

c)
RFP postive cells (%)
100
75
50
25
0
0
20
40
80
160
320
640
[RFP mRNA] ng/mL

Fig. 3A

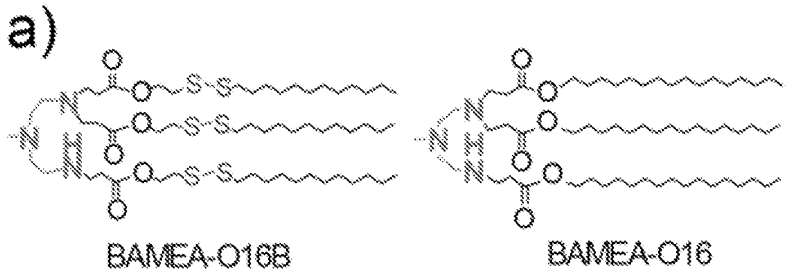

a)
BAMEA-O16B
BAMEA-O16

Fig. 3B

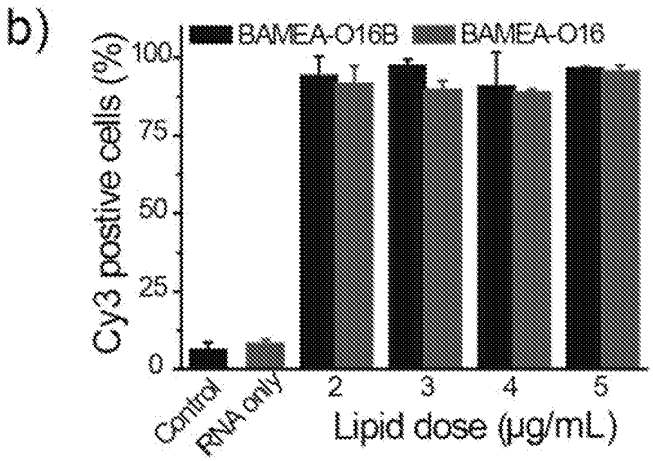

b)
BAMEA-O16B
BAMEA-O16
Cy3 postive cells (%)
100
75
50
25
0
Control
RNA only
2
3
4
5
Lipid dose (μg/mL)

Fig. 3C

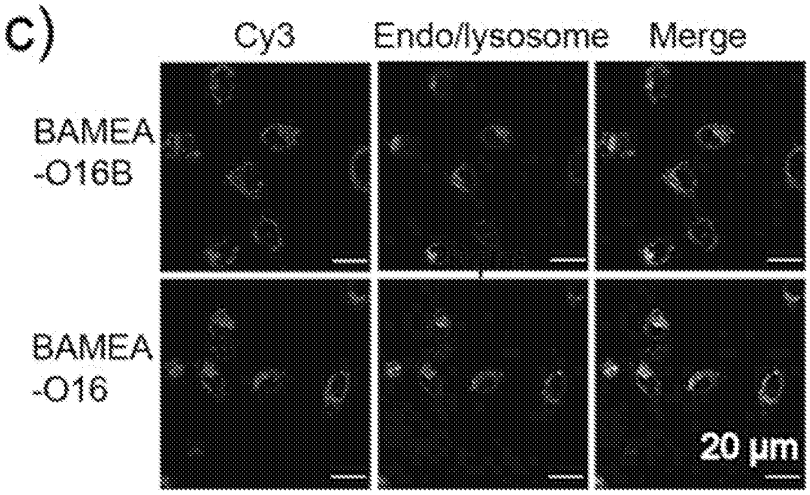

c)
Cy3
Endo/lysosome
Merge
BAMEA
-O16B
BAMEA
-O16
20 µm

Fig. 3D

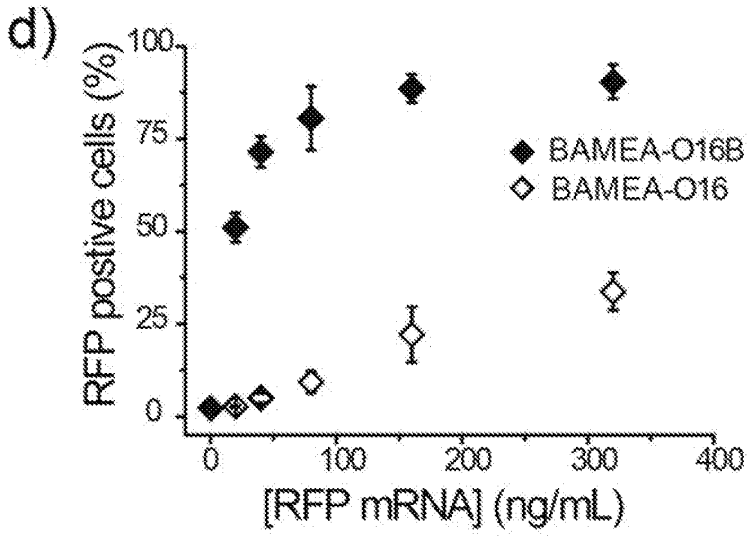

d)
RFP postive cells (%)
100
75
50
25
0
0
100
200
300
400
[RFP mRNA] (ng/mL)
BAMEA-O16B
BAMEA-O16

Fig. 4A

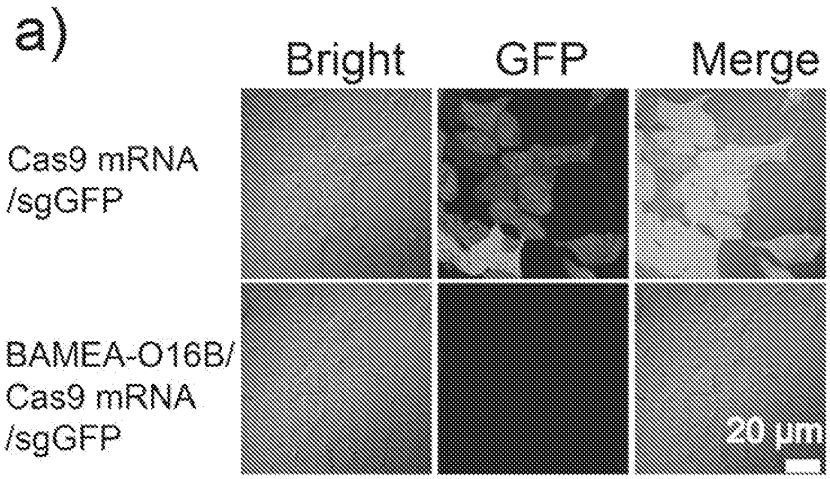

a)
Bright
GFP
Merge
Cas9 mRNA /sgGFP
BAMEA-O16B/ Cas9 mRNA /sgGFP
20 μm

Fig. 4B

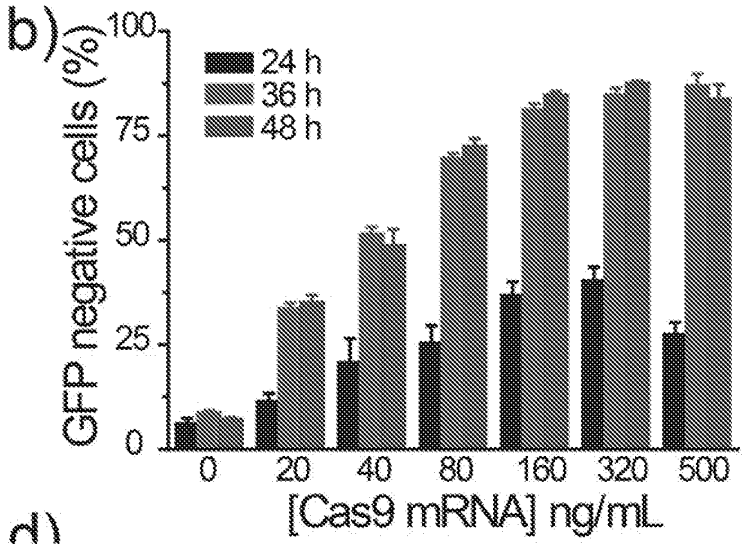

b)
GFP negative cells (%)
100
75
50
25
0
24 h
36 h
48 h
0
20
40
80
160
320
500
[Cas9 mRNA] ng/mL

Fig. 4C

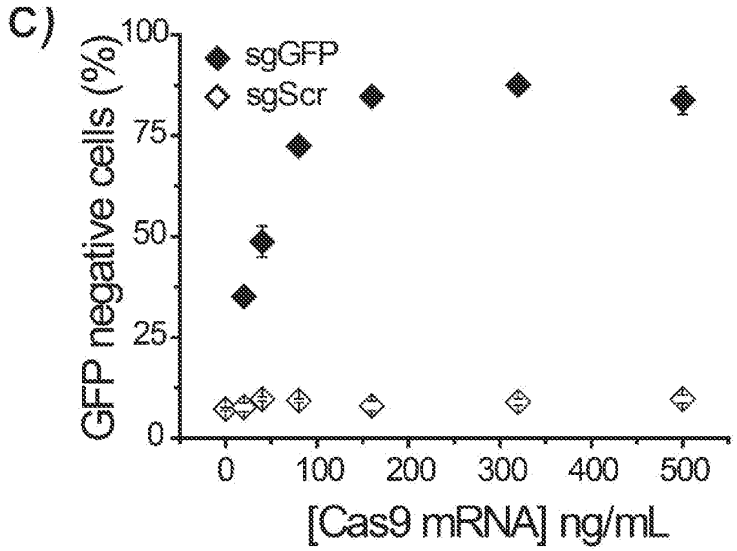

c)
GFP negative cells (%)
100
75
50
25
0
sgGFP
sgScr
0
100
200
300
400
500
[Cas9 mRNA] ng/mL

Fig. 4D

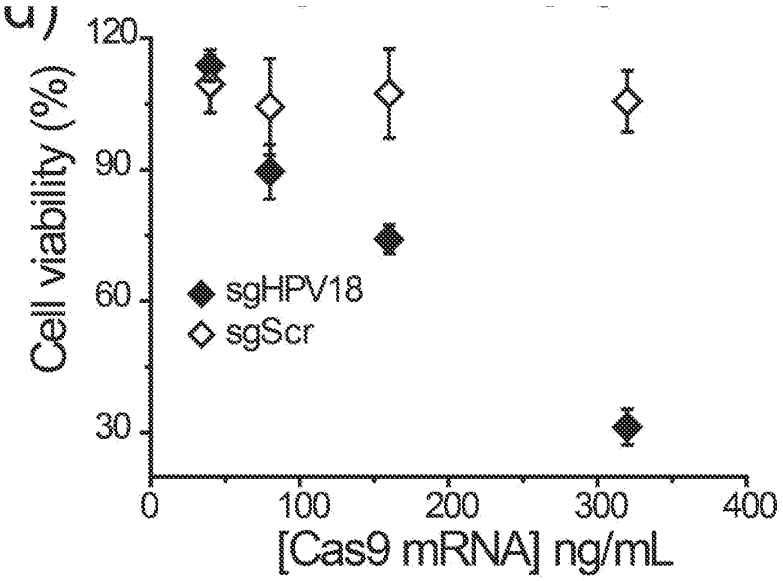

d)
Cell viability (%)
120
90
60
30
sgHPV18
sgScr
0
100
200
300
400
[Cas9 mRNA] ng/mL

Fig. 5A

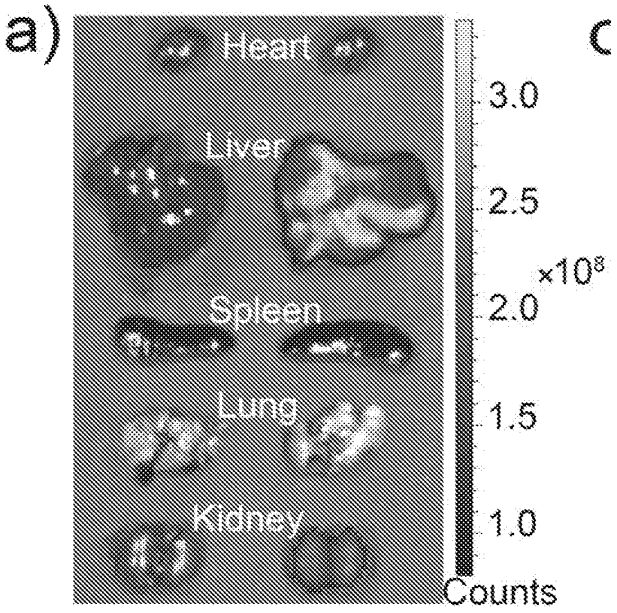

a)
Heart
Liver
Spleen
Lung
Kidney
3.0
2.5
2.0
1.5
1.0
×10⁸
Counts

Fig. 5B

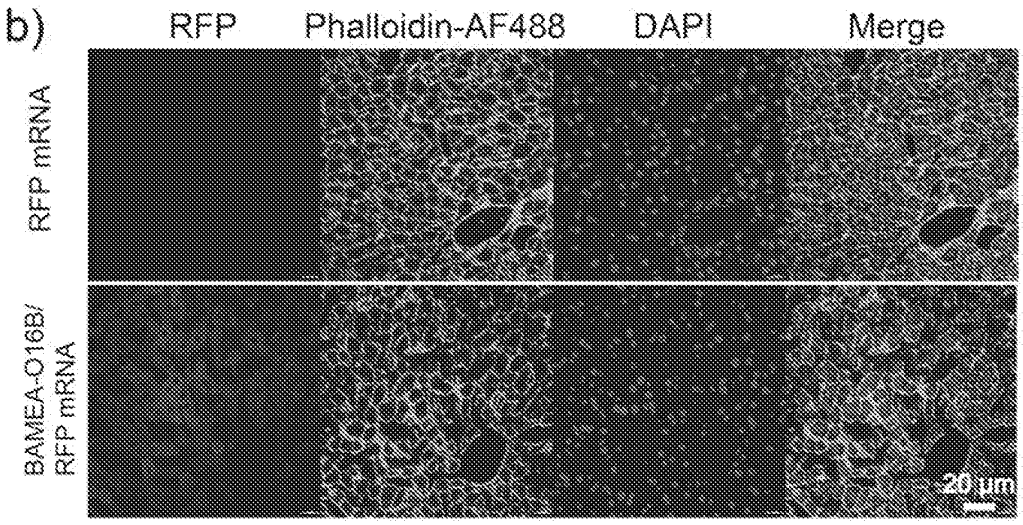

b)
RFP
Phalloidin-AF488
DAPI
Merge
RFP mRNA
BAMEA-O16B/
RFP mRNA
20 um

Fig. 5C

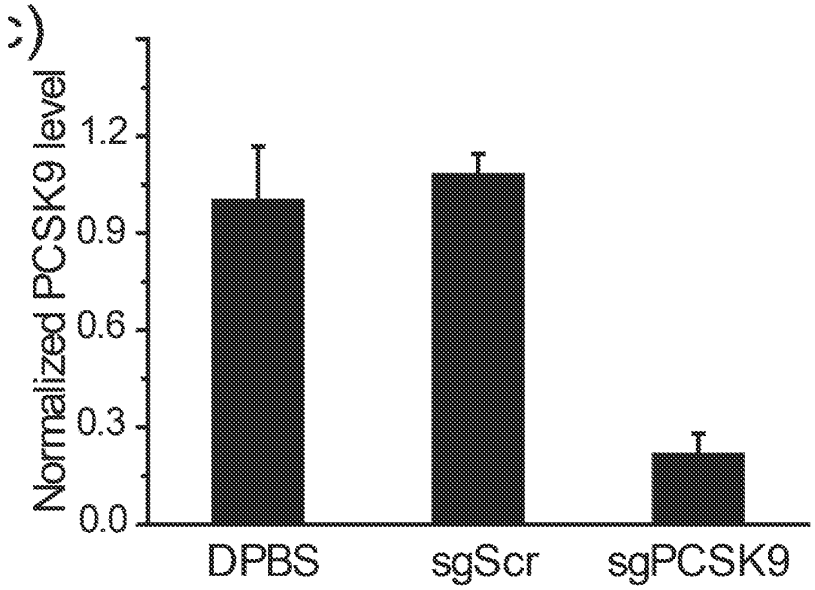


Fig. 6A

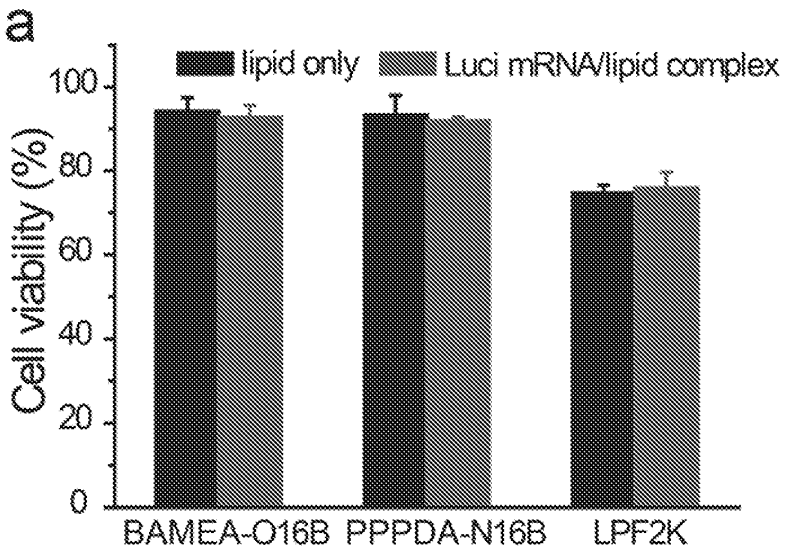

a
lipid only
Luci mRNA/lipid complex
Cell viability (%)
100
80
60
40
20
0
BAMEA-O16B
PPPDA-N16B
LPF2K

Fig. 6B

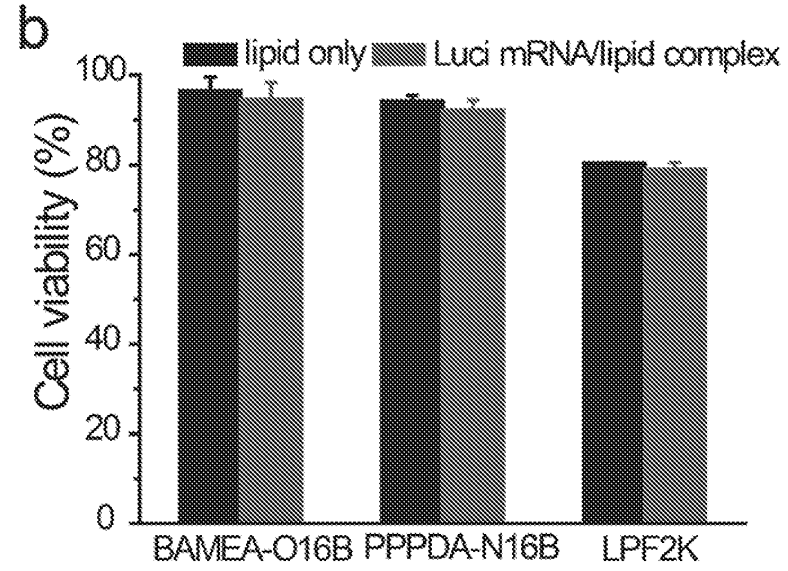
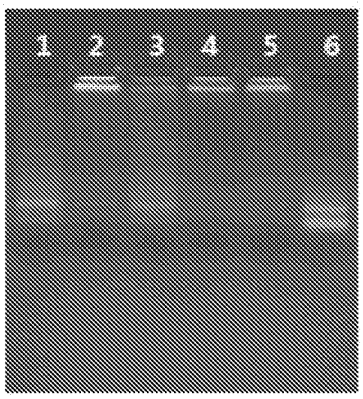

b
lipid only
Luci mRNA/lipid complex
Cell viability (%)
100
80
60
40
20
0
BAMEA-O16B
PPPDA-N16B
LPF2K

Fig. 7



lane 1: RFP mRNA
lane 2: RFP mRNA + BAMEA-O16B
lane 3: RFP mRNA + BAMEA-O16B + GSH
lane 4: RFP mRNA + BAMEA-O16
lane 5: RFP mRNA + BAMEA-O16 + GSH
lane 6: RFP mRNA + GSH

Fig. 8

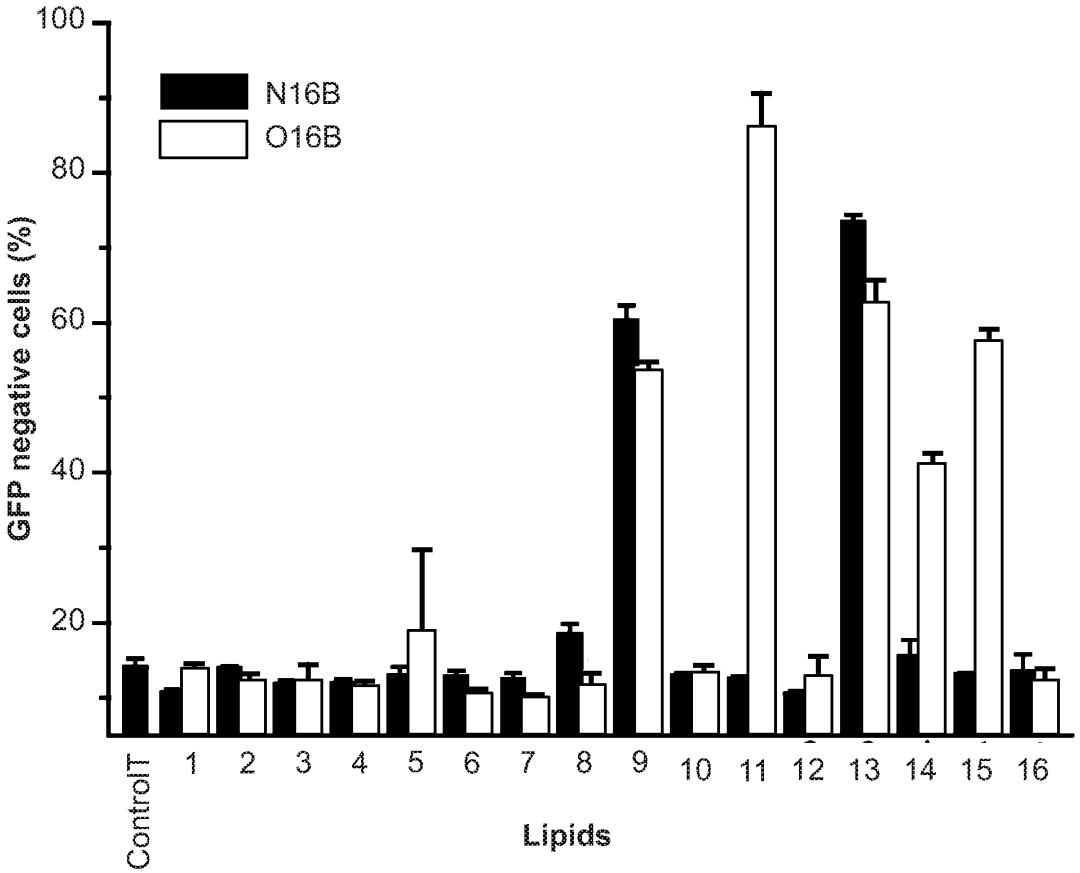


Fig. 9

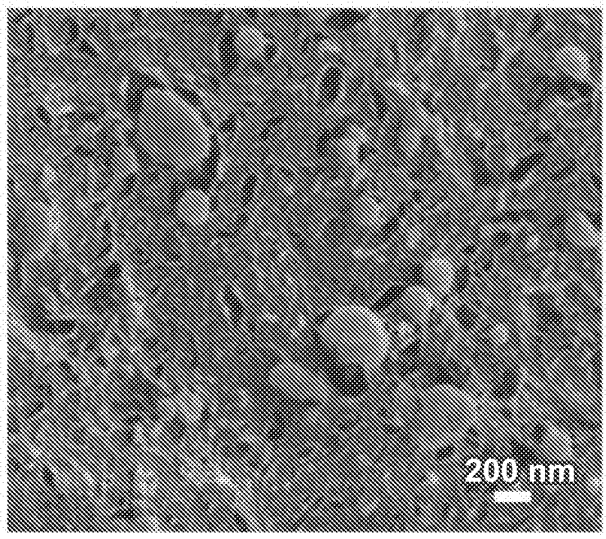

200 nm

Fig. 10

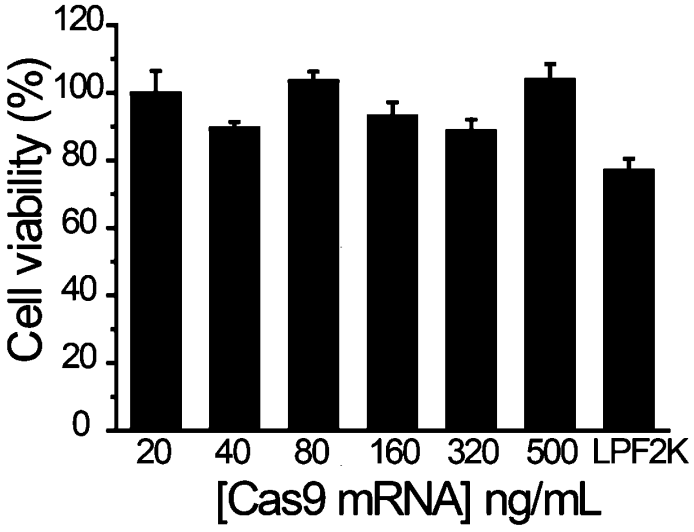

Cell viability (%)
120
100
80
60
40
20
0
20
40
80
160
320
500
LPF2K
[Cas9 mRNA] ng/mL

Fig. 11

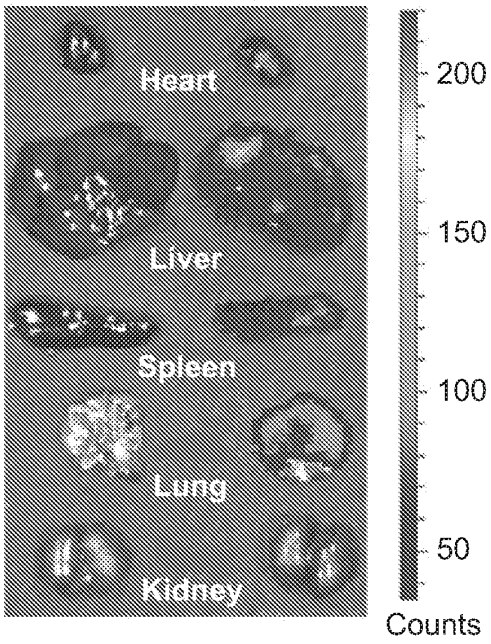

Heart
Liver
Spleen
Lung
Kidney
200
150
100
50
Counts

Fig. 12

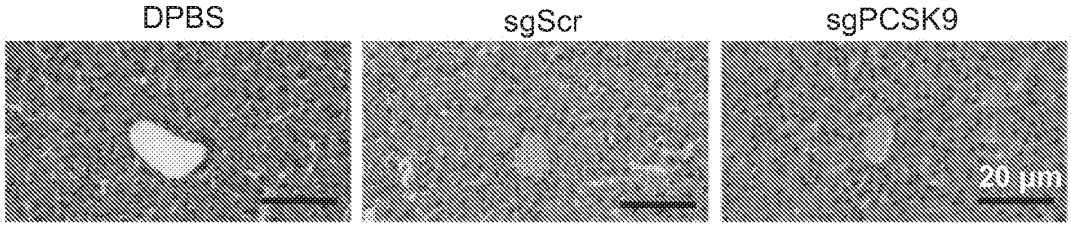

DPBS
sgScr
sgPCSK9
20 μm

Fig. 13

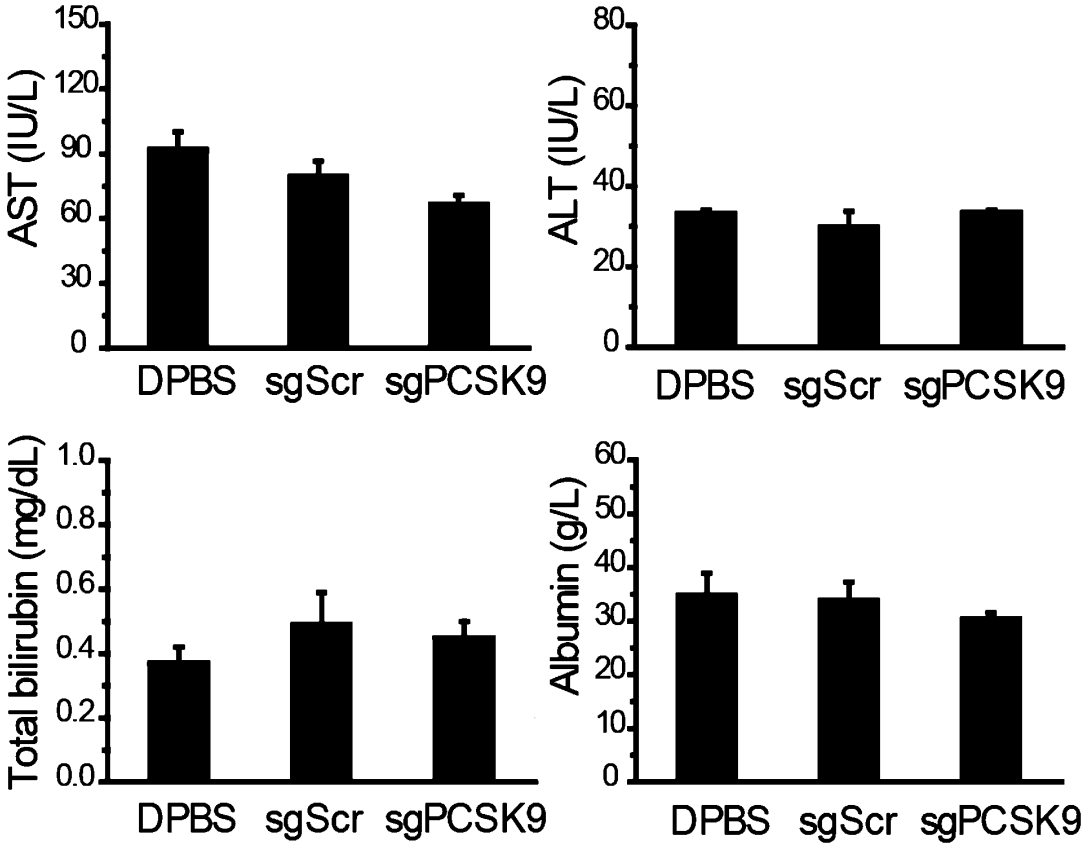

AST (IU/L)
150
120
90
60
30
0
DPBS
sgScr
sgPCSK9
ALT (IU/L)
80
60
40
20
0
DPBS
sgScr
sgPCSK9
Total bilirubin (mg/dL)
1.0
0.8
0.6
0.4
0.2
0.0
DPBS
sgScr
sgPCSK9
Albumin (g/L)
60
50
40
30
20
10
0
DPBS
sgScr
sgPCSK9

Fig. 14

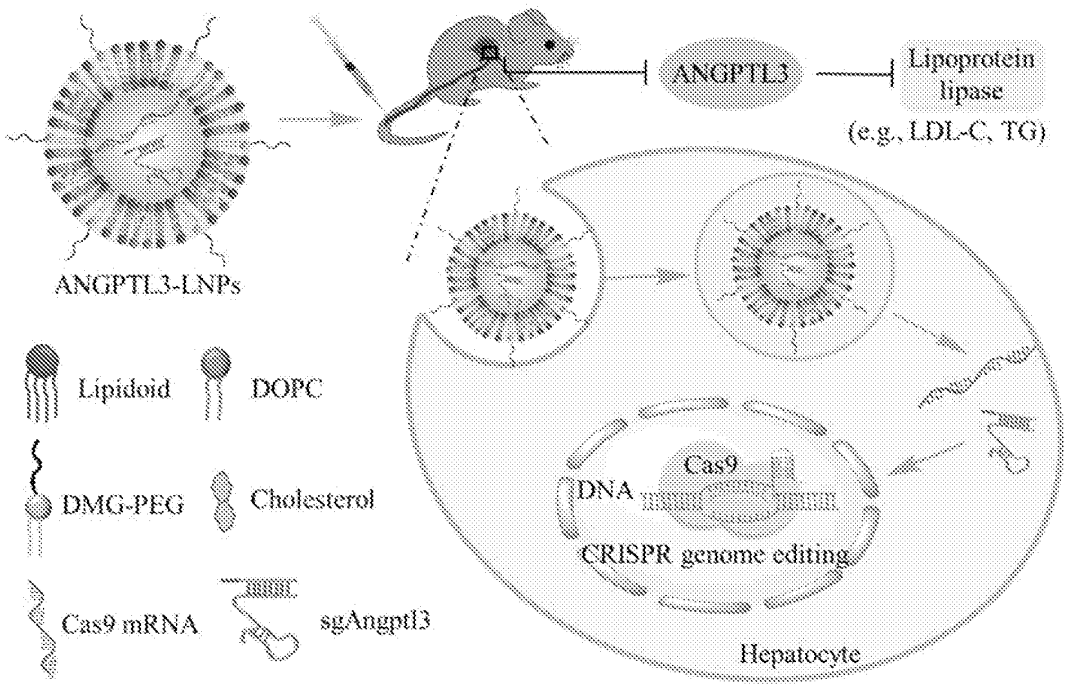

ANGPTL3
Lipoprotein lipase
(e.g., LDL-C, TG)
ANGPTL3-LNPs
Lipidoid
DOPC
DMG-PEG
Cholesterol
Cas9 mRNA
sgAngptl3
Cas9
DNA
CRISPR genome editing
Hepatocyte

Fig. 15A

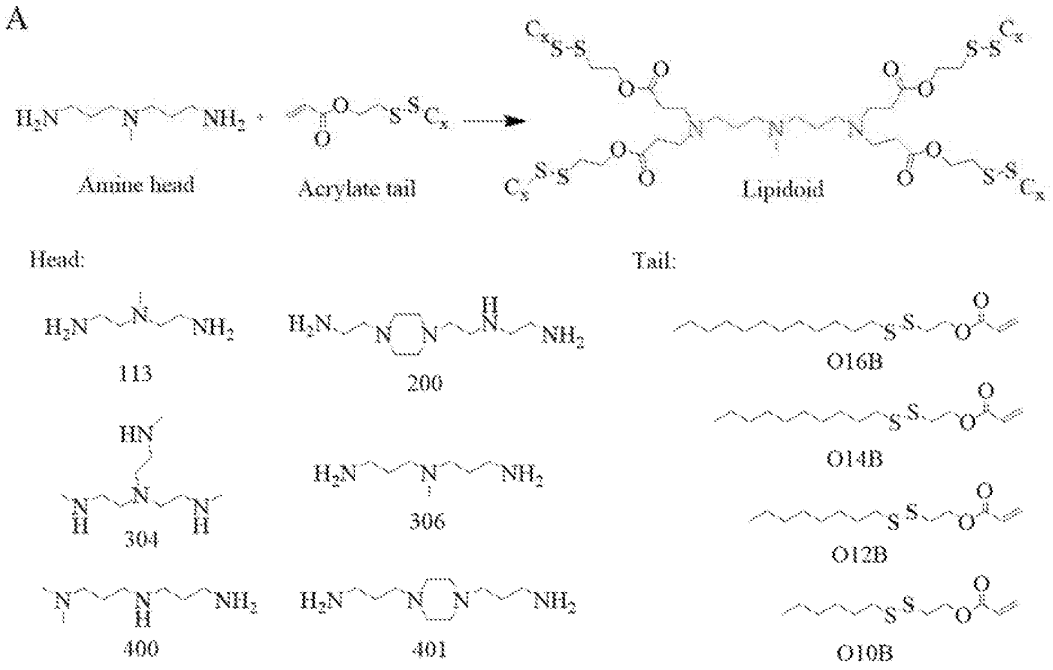


Fig 15B

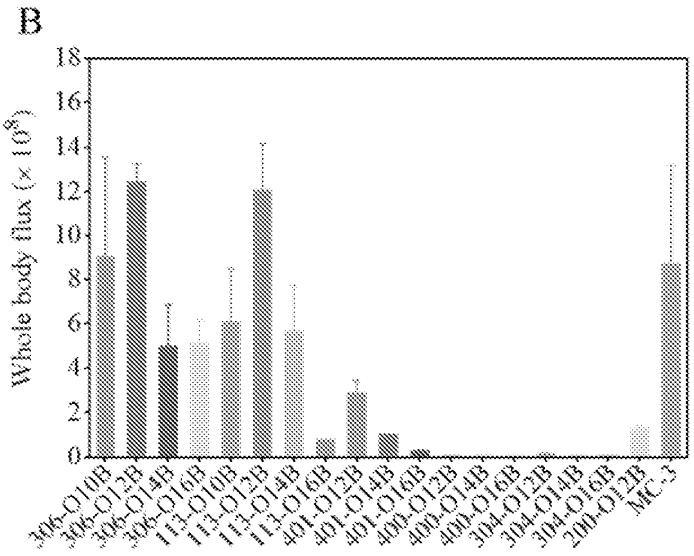


Fig. 15C

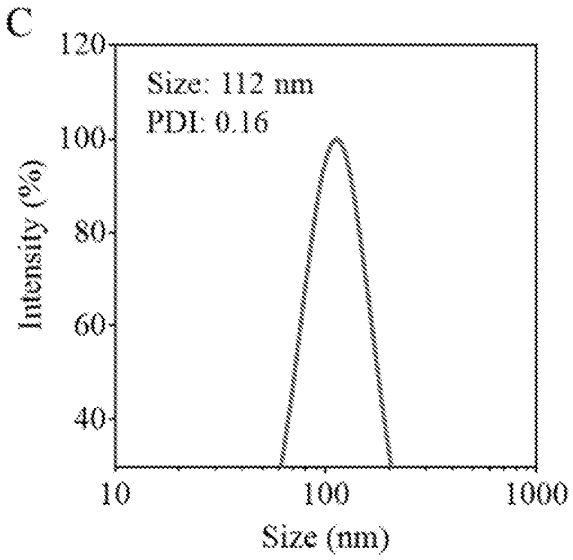


Fig. 16A

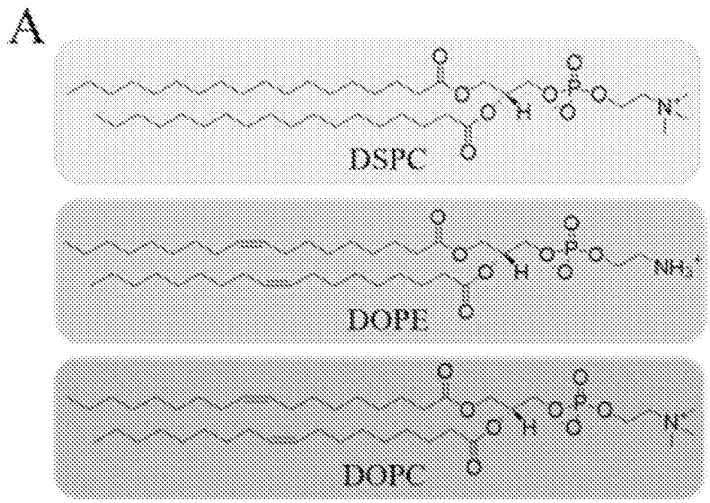


Fig. 16B

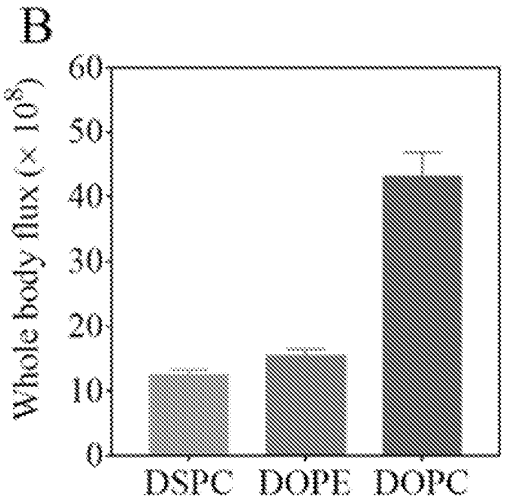


Fig. 16C

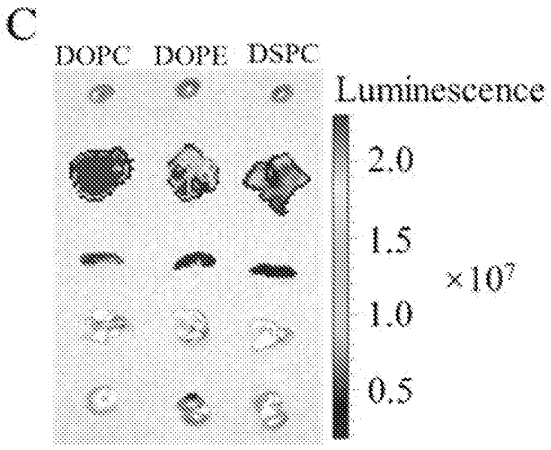


Fig. 16D

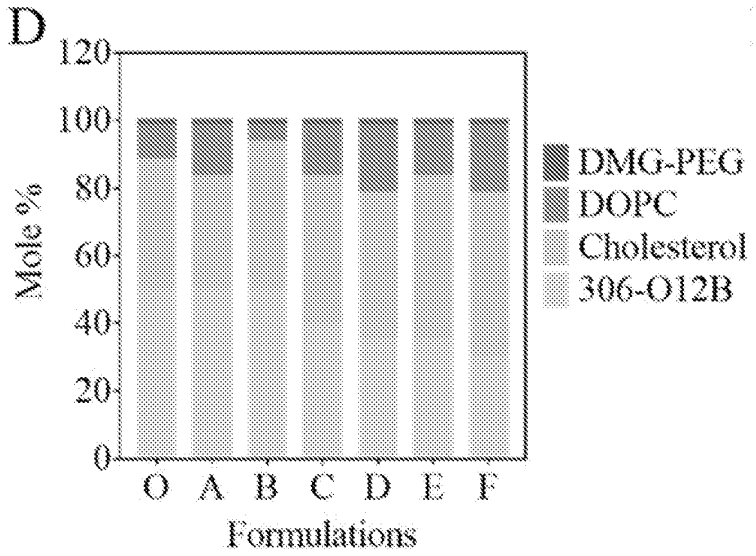


Fig. 16E

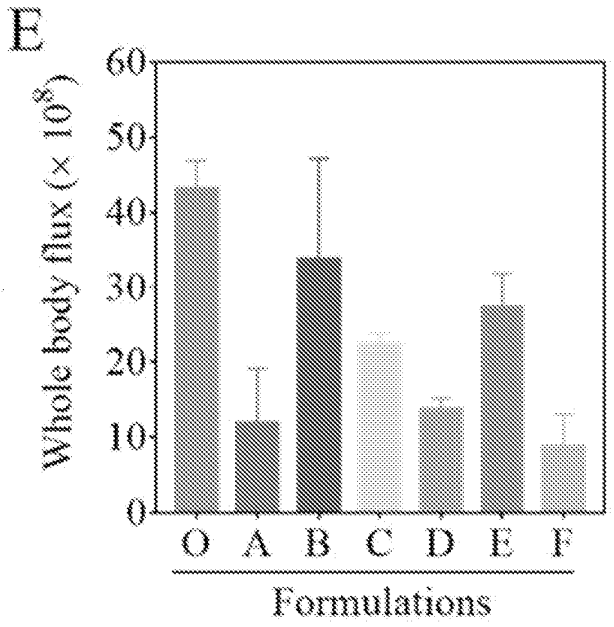


Fig. 16F

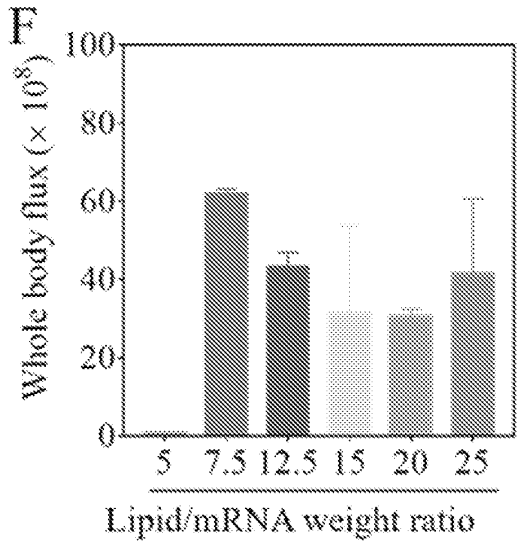


Fig. 17A

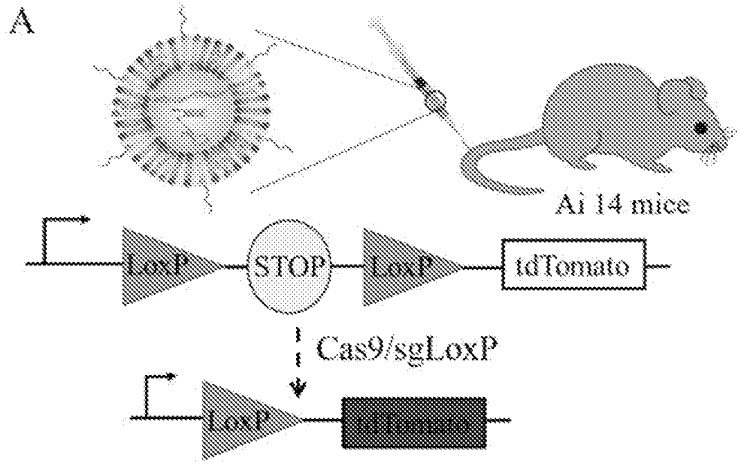


Fig. 17B

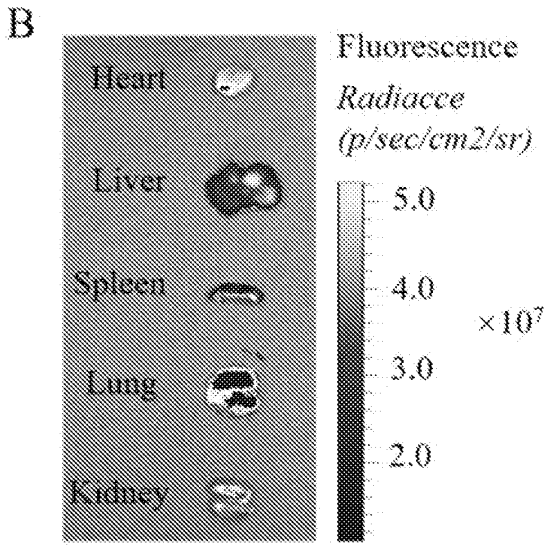


Fig. 17C

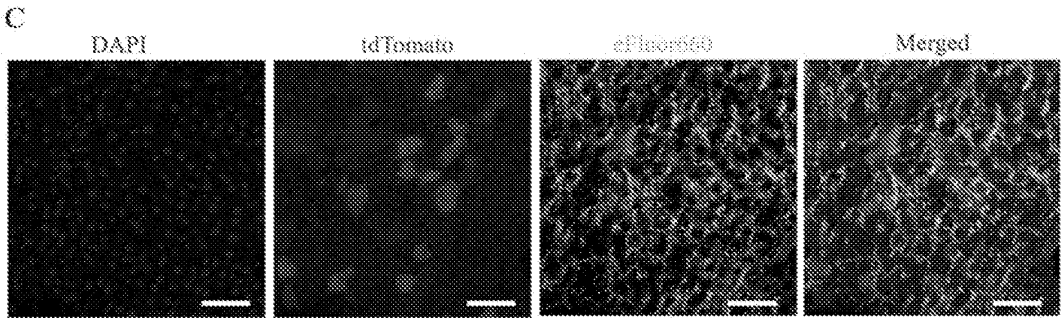


Fig. 18A

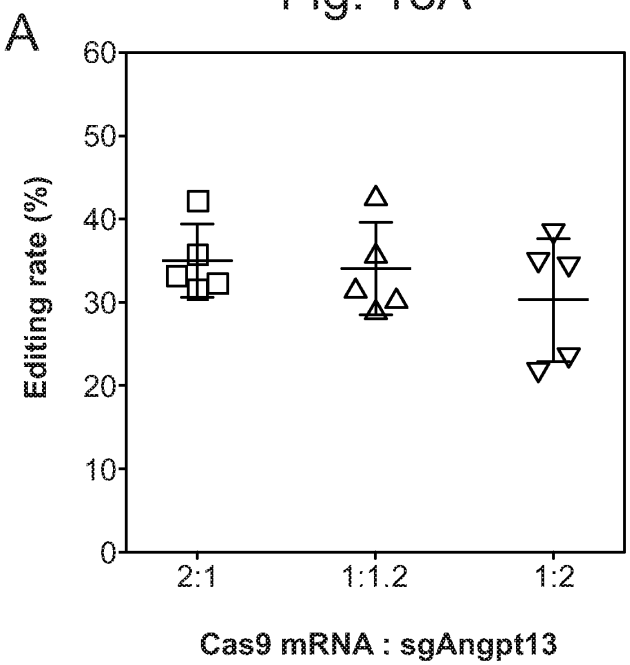


Fig. 18B

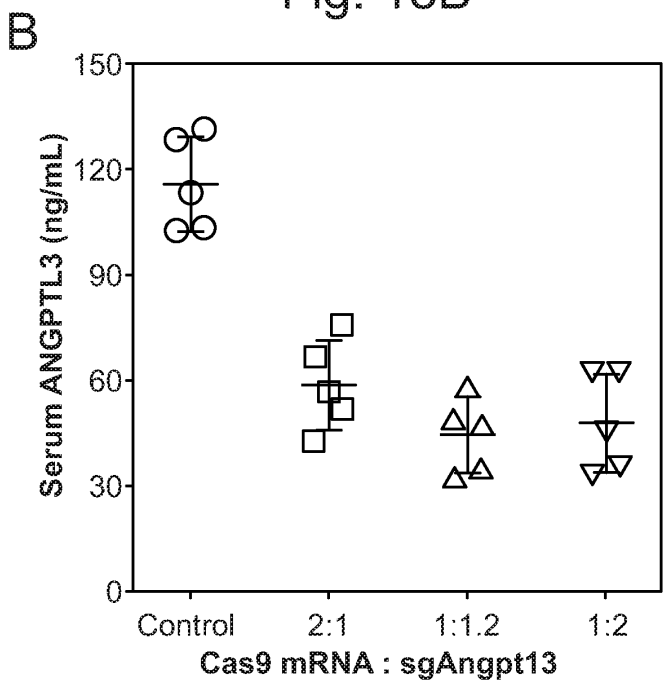


Fig. 19A

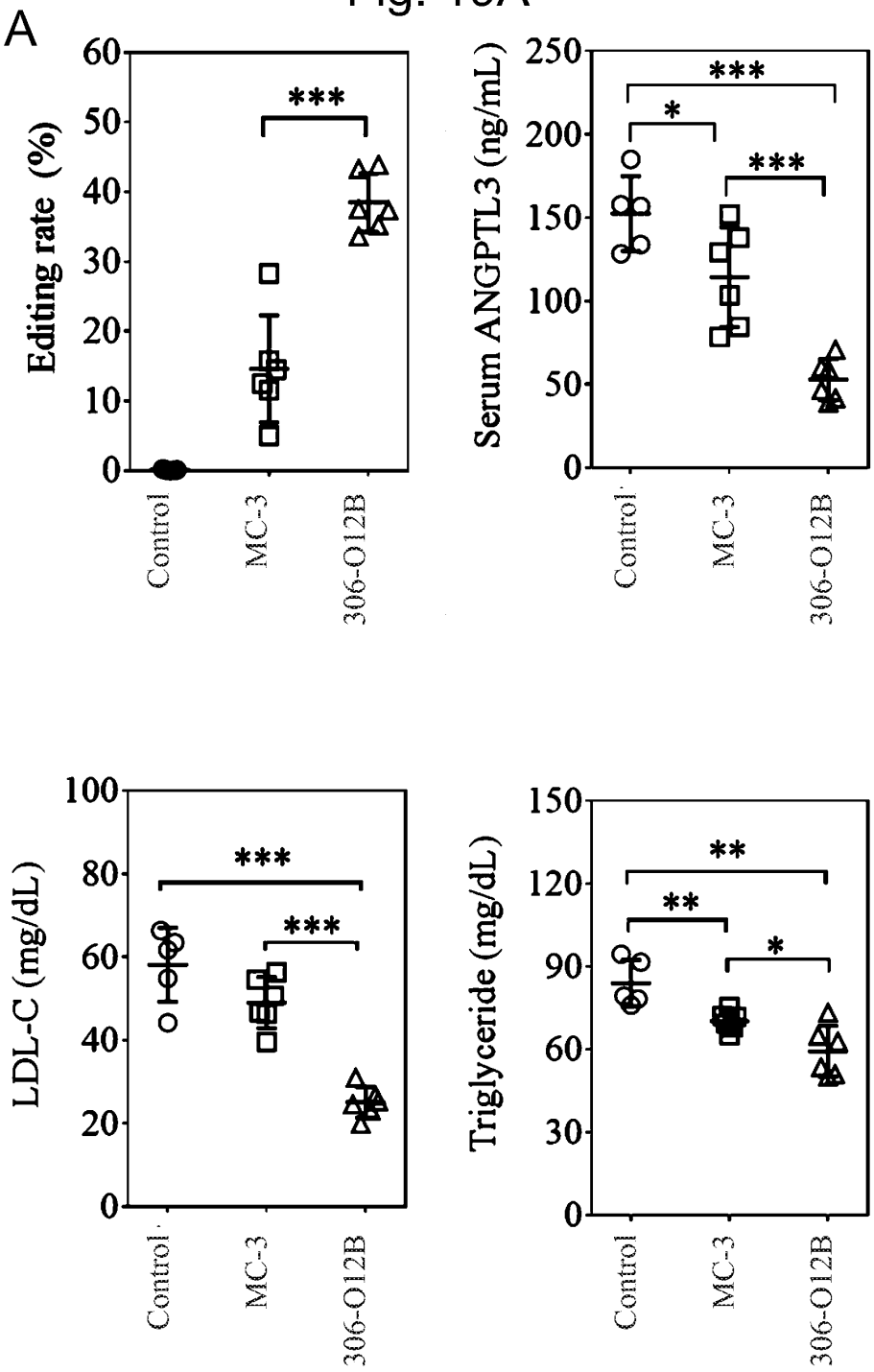

A
Editing rate (%)
60
50
40
30
20
10
0
***
Control
MC-3
306-O12B
Serum ANGPTL3 (ng/mL)
250
200
150
100
50
0
***
*
***
Control
MC-3
306-O12B
LDL-C (mg/dL)
100
80
60
40
20
0
***
***
Control
MC-3
306-O12B
Triglyceride (mg/dL)
150
120
90
60
30
0
**
**
*
Control
MC-3
306-O12B

Fig. 19B

B

Edited ANGPTL3 sequence	Mutation type	frequency in best 306-O12B mouse (%)	frequency in best MC-3 mouse (%)	frequency in control mouse (%)
CGTTTTTAACTTGTAGTGTA	wild-type			
CGTTTT_AACTTGTAGTGTA	1nt deletion	31.0	20.0	0.19
CGTTTTT|AACTTGTAGTGTA T	1nt insertion	12.0	8.0	0
CGTTT__AACTTGTAGTGTA	2nt deletion	2.0	1.0	0
other edits		3.4	0.9	0.11
total edited alleles		48.4	29.9	0.3

Fig. 19C

C

Potential off-target site	Location	frequency in best 306-O12B mouse (%)	frequency in best MC-3 mouse (%)	frequency in control mouse (%)
aACACTACAA-TTAAAAAACGTGG	chr17: 67139471	0.06	0.04	0.06
TACACTAaAA-TTAAAAAACGTGG	chr10: 96372376	0.03	0.05	0.05
TtCACTACAAGTTAGAAAAACaGGG	chr3: 96010269	0.03	0.03	0.03
TACACTACGAAAaTTAAAAAcCGAGG	chr14: 113652925	0.04	0.04	0.02
TACACTACAAGTTCAAAtACaTGG	chr9: 20240345	0.02	0.04	0.02
TACACTA-AAGaTAAAAAACaTGG	chr8: 57778609	0.04	0.04	0.04
TACAgT-CAAGTTAAAAAACcAGG	chr12: 53110237	0.09	0.09	0.09
T-CACTACtAGaTAAAAAACGTGG	chr3: 65249939	0.04	0.04	0.03
cACACTACAAG-TAAAAAACaGGG	chr3: 38440103	0.04	0.05	0.04

Fig. 20A

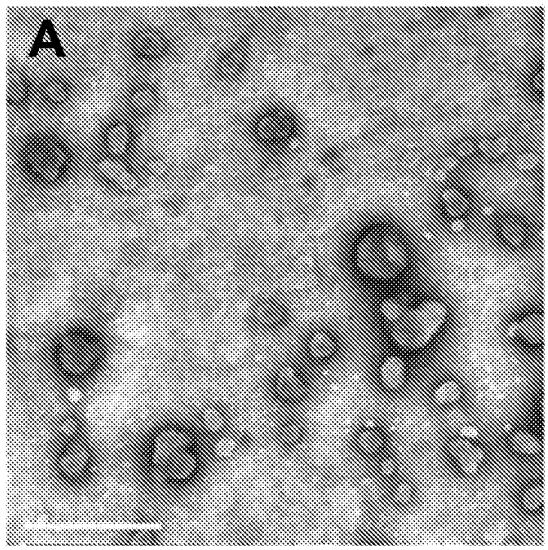


Fig. 20B

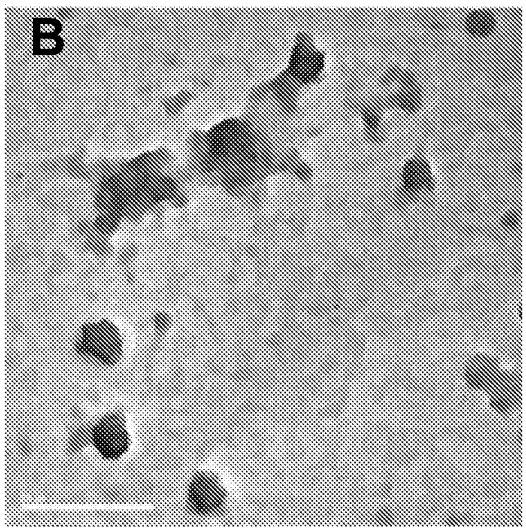


Fig. 21

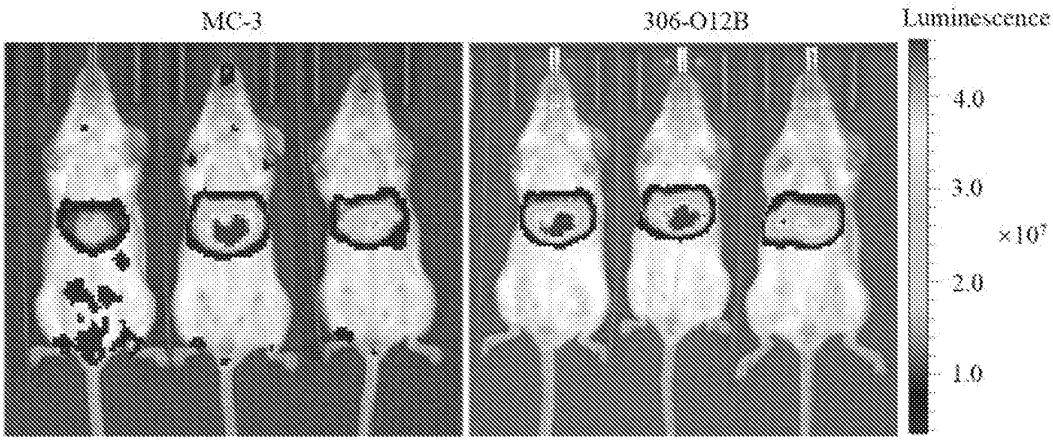


Fig. 22

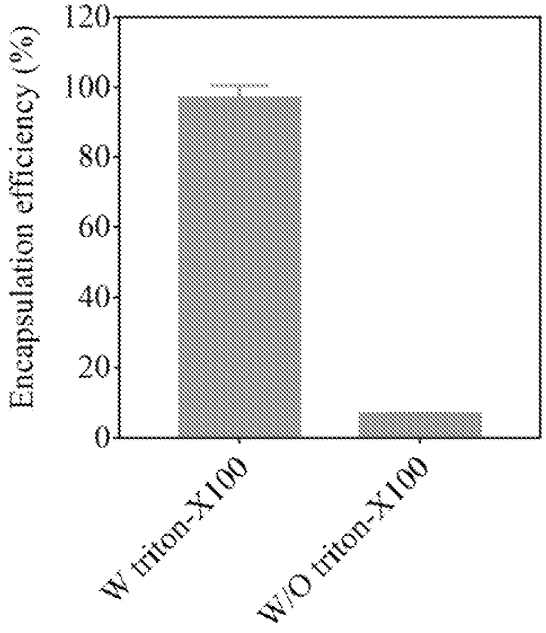


Fig. 23A

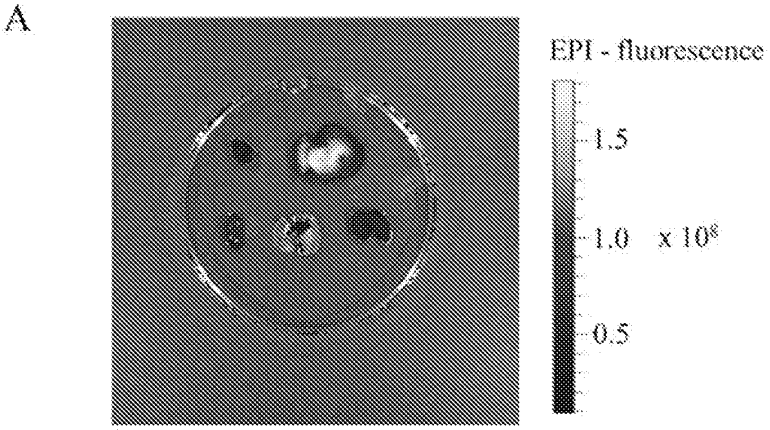


Fig. 23B

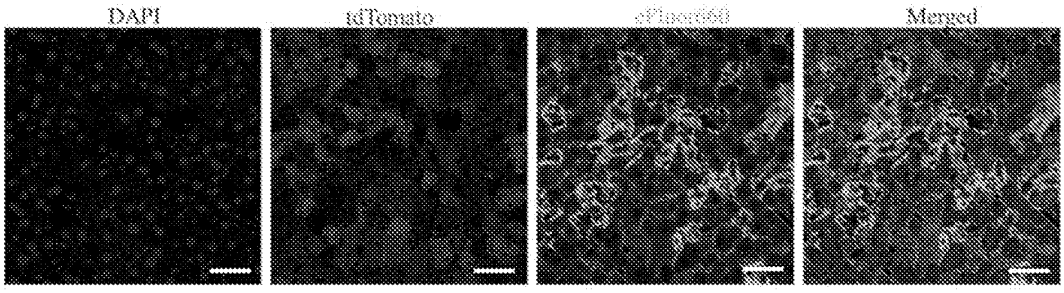


Fig. 24

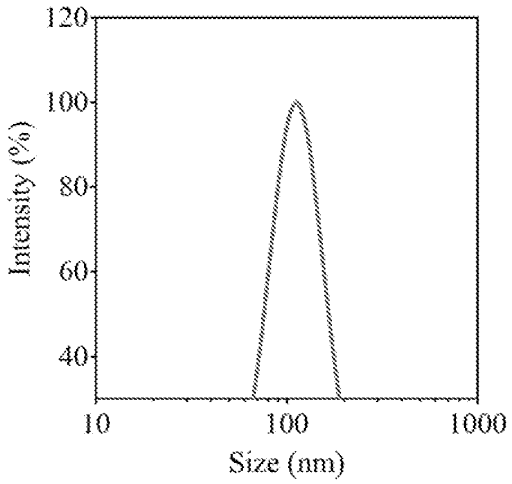


Fig. 25

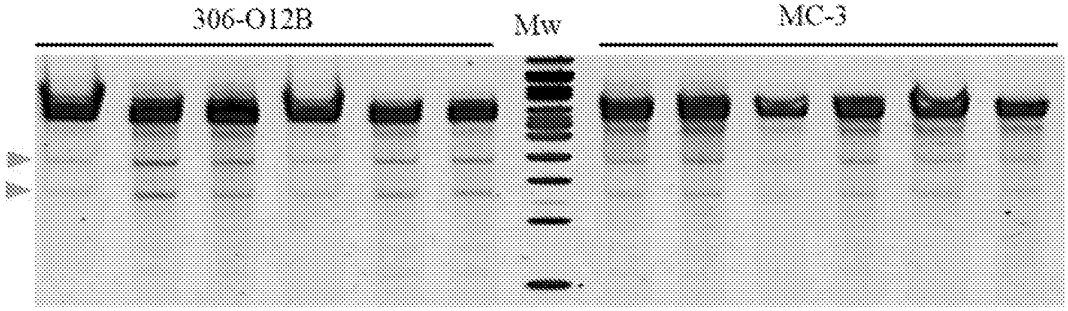
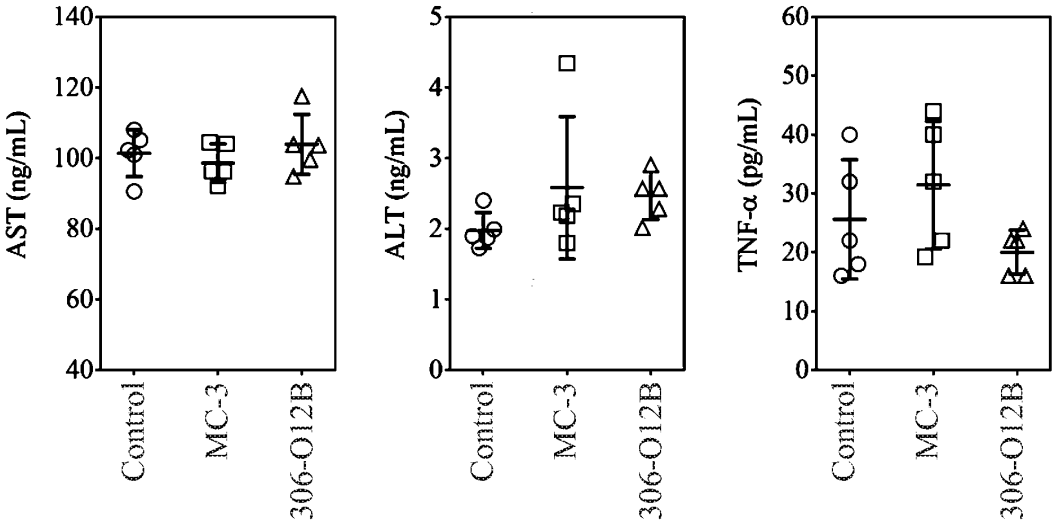


Fig. 26


AST (ng/mL)
140
120
100
80
60
40
Control
MC-3
306-O12B
ALT (ng/mL)
5
4
3
2
1
0
Control
MC-3
306-O12B
TNF-α (pg/mL)
60
50
40
30
20
10
0
Control
MC-3
306-O12B